1/28/2002 D.D.

Plasmid Prep, PCR, and PCR Clean-up with Qiagen Robot

Growing Cultures

1. Thaw master plates about 20-30 min. until slushy on the benchtop. Don't entirely dethaw!
2. Fill Qiagen Flat Bottom culture blocks with 1.3ml of LB media to which 1.25ml of ampicilan has been added to a full bottle (500 mL) of LB.
3. Using metal 96 tip replicator transfer block and 100% ethanol, flame sterilize replicator, place into master plates all the way to the bottom, then place into culture blocks without touching sides of blocks. Cover with aluminum foil.
4. Flame replicator again and repeat for all samples.
5. Incubate overnight at 37°C, 200 rpm on platform shaker for 16-22 hours.
6. Refreeze master plates.

Plasmid Prep

1. Next day, check culture blocks for growth. Note which wells are cloudy and which are not.
2. Centrifuge blocks at 3000rpm for 5 min. If media is still cloudy, centrifuge more.
3. Remove supernatant by decanting. Invert the block over the sink to remove used broth. Blot 2-3 times on absorbent paper.
4. Turn on Qiagen computer (robot should remain on at all times!). There is no sign-on password, just hit okay. Start QIAsoft 4.1. Program should start in the Execute environment. On the drop down menu next to the Run button, select your program.
   -If you only have 96 samples or less, select: Vector DNA Applications and Plasmid purification (96 samples or less).
   -If you have more than 96 samples, under the drop down menu select: Vector DNA Applications and Plasmid purification (96 to 384 samples).
5. Click the run button to start the program and follow prompts to set up samples and reagents. Computer will beep and stop when a user interaction is required.
6. When run is complete, if no more samples are to be processed, run the wash program (program will prompt you for it). (Note: under Accessory Applications the Wash program can also be selected to run.) Cover and label samples and place in 4°C fridge until ready to run PCR.

PCR

1. Turn on PCR machine. Follow charts for amounts of reagents and primer types.
2. Place 1 µl of plasmid into a 96well PCR plate with Matrix tips(return tips to box for reuse). Combine all reagents for PCR. Add Taq last to reagent pool and distribute 99 µl into each well with yellow 100µl tips.
3. Run appropriate PCR program. (rat uses the Human program)
4. While PCR is running, prepare a 2% agarose gel. One gel will run two 96well plates worth of samples. Mix 5 grams of agarose with 250ml 1xTBE buffer. Heat until dissolved (about 2x's at 2 min.).
5. Let cool to 55°C (too hot warps gel box) and add 25 µl EtBr and swirl.
6. Pour gel and add combs (4/gel) and allow to solidify, about 45-60 min.
7. Place solid gel in gel box containing enough 1xTBE to cover top of gel.

8. Once PCR is done running, take 2µl of PCR sample plus 4µl loading dye and load into gel. (loading will be in a staggered pattern). Use Matrix tips saved from running PCR. Place remaining PCR products in 4°C fridge. In first and last lanes of each row on gel add 4µl of 100bp ladder.
9. Run gel at about 85 volts for 25-30 minutes or until dye is halfway.
10. View gel and save image on zip disk and print a copy. If products look good, proceed to PCR cleanup.

PCR Clean-up

1. Turn on Qiagen computer and select program. The program is different depending on which buffers are included in the kit. (The only differences are the buffer concentrations and amounts used.)
   -If running 96 samples or less with Buffer **PB**, select: PCR Applications and PCR Purification_QIAquick (up to 96 samples).
   -If running 96 samples or less with Buffer **PM**, select: PCR Applications and PM_ PCR Purification_QIAquick (up to 96 samples).
   -If running more than 96 samples with Buffer **PB**, select : PCR Applications and PCR Purification_QIAquick (96 to 384).
   -If running more than 96 samples with Buffer **PM**, select : PCR Applications and PM_PCR Purification_QIAquick (96 to 384).

Follow prompts from computer. Use micro plates included in kit to elute samples into.

2. When complete, if no more samples are to be processed, allow the wash program to run.
3. Transfer by hand the eluted samples into 96well costar V-bottom plates.
4. Place samples in costar plates in speed vac and turn on until samples are dry, about 2 hours (don't overdry and don't turn on centrifuge).
5. Add 25µl of 1xArrayIt and mix well. Place in 37°C incubator overnight in a ziplock with wet paper towels.
6. Next day transfer to -80°C freezer for storage.

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PCR Info.

PCR Program: Human (for Human, Rat and Mouse)

Phase 1	1 cycle	94°C, 30 sec
Phase 2	35 cycles	94°C, 30 sec
		54°C, 30 sec
		72°C, 150 sec
Phase 3	1 cycle	72°C, 5 min
		4°C, ∞

PCR Reagents

10X Buffer (1ml/tube)
MgCl (1ml/tube)
dNTP Pre-mixed Perkin Elmer
sterile dH2O
Taq Polymerase (100µl/tube)

Human – M13 variants

(ul's)	1 sample	1 plate	2 plates	3 plates	4 plates
10X buffer	10	1100	2100	3100	4100
MgCl	3	330	630	930	1230
100mM dNTP's	0.8	88	168	248	328
H2O	86.8	9548	18228	26908	34522
500uM F primer	0.2	22	42	62	82
500uM R primer	0.2	22	42	62	82
Taq	1	110	210	310	410
Total	100	11000	21000	31000	41000

Rat (also Mouse) - Research Genetics primers GF200

(ul's)	1 sample	1 plate	2 plates	3 plates	4 plates
10X buffer	10	1100	2100	3100	4100
MgCl	3	330	630	930	1230
100mM dNTP's	0.8	88	168	248	328
H2O	85	9350	17850	26350	34600
1mM F primer	0.1	11	21	31	41
1mM R primer	0.1	11	21	31	41
Taq	1	110	210	310	410
Total	100	11000	21000	31000	41000